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PERFORMANCE OF IMMOBILIZED BACTERIAL ALPHA-AMYLASE IN METHYLTRIETHOXYSILANE/ TETRAETHOXYSILANE SOL-GEL MATRICES

Beatrice Vlad-Oros[a], O. Oniga[a], Z. Dudas[a], Monica Dragomirescu[b], Gabriela Preda[a], A. Chiriac[a]

[a]West University of Timişoara, Faculty of Chemistry, Biology, Geography, Department of Chemistry, Pestalozzi, 16, Timişoara, 300115, Romania

[b]Banat University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and Biotechnologies, Calea Aradului, 119, Timişoara, 300645, Romania

SUMMARY

The large number of studies related to the field of biomolecules encapsulation in sol-gel hosts clearly indicates that this approach can be considered as a powerful alternative to traditional encapsulation procedures involving biopolymer hosts. In this study, α-amylase was immobilized, by using the sol-gel technique, in silica particles obtained from hydrolysis and polycondensation of tetraethoxysilane (TEOS) and a mixture of methyltriethoxysilane (MTES) and tetraethoxysilane. The influence of the pH and temperature of free and immobilized α-amylase were compared. It was shown that the relative activities of immobilized enzymes are higher than those of free enzymes over broader pH and temperature ranges. The Michaelis constant and the maximum rate of starch hydrolysis reaction were calculated by fitting the experimental data to the Michaelis-Menten equation. It was found that K_M and V_{max} values of the immobilized enzyme were smaller than those of the free enzyme.

Keywords: α-amylase, tetraethoxysilane, methyltriethoxysilane, sol-gel, entrapment, kinetics.

INTRODUCTION

The sol-gel process provides a unique opportunity to immobilize biomolecules and cells into stable matrices with tunable structural properties, while fully maintaining their characteristics. The possibility to obtain such hybrid materials in a wide variety of dimensions and shapes allows their application in various fields [1, 2].

α-Amylase (E.C. 3.2.1.1), endoenzyme hydrolyzing α-1,4 linkages, is marketed with applications in different domains (sugar, textile, brewery industry), one of its most important applications concerning the liquefaction of starch to oligosaccharides that can be further converted by glucoamylases to produce sugars that can be used during fermentation [3, 4].

Enzyme immobilization has been reported to improve the stability of enzymes and may also affect binding of substrates to the enzymes, thereby affecting the Michaelis constant [5, 6].

In this study, α-amylase was immobilized, by using the sol-gel technique, in silica matrices obtained from tetraethoxysilane (TEOS) and a mixture of methyltriethoxysilane (MTES) and tetraethoxysilane. The optimal conditions for substrate hydrolysis – pH, temperature and kinetic parameters of these biomaterials were studied and compared with those of the free enzyme.

MATERIALS AND METHODS

Bacterial α-amylase (E.C. 3.2.1.1) was obtained from Fluka. Soluble starch was purchased from Bender&Hobein and Merck. Methyltriethoxysilane (MTES) and tetraetoxysilane (TEOS) were from Aldrich. All other chemicals were of analytical grade and were used without further purification.

The immobilization of enzyme was performed in two different ways:

1. A silica sol was obtained from TEOS, ethanol and water (1.25 : 1.25 : 1, v/v), in acid catalysis (HCl 1N). Then the sol was mixed with ethanol and water (1.33 : 1.33 : 1, v/v), 5 drops NH_3 12% and 1.25 mL buffered enzymatic solution, containing 25 mg α-amylase. The gelation occurred in a few minutes.

2. Hybrid matrices were prepared using tetraethoxysilane (TEOS) and methyltriethoxysilane (MTES) in different molar ratios (1:1, 2:1 and 3:1, respectively). The mixture containing the alkoxides, ethanol, water (3 : 2.5 : 1, v/v) and 11 μL HCl (0.04 N) was stirred for an hour. Then 0.94 mL buffered enzymatic solution, containing 0.019 mg α-amylase and 100 μL NaF 1M are added under stirring [7].

The gels were left overnight for aging, washed and dried.

The activity of α–amylase was spectrophotometrically assayed by using soluble starch as substrate (Hitachi 1100 spectrophotometer).

Residual starch concentration assay (I_2/I^-): 0.5 mL soluble starch (0.4%), 0.4 mL phosphate buffer (0.05 M, pH 5.2) and 0.1 mL enzymatic solution or 0.01 g immobilized biocatalyst were kept for 5 min. at 25ºC. 5 mL solution I_2/I^- M/1000 and 15 mL distilled water were added. The absorbance was measured at 595 nm against distilled water. One unit of α-amylase activity was defined as the amount of enzyme required to hydrolyze 1 mg starch in 5 min at 25°C when 2 mg starch was present at the start of the reaction.

The effect of the temperature on the activity of native and immobilized enzyme was estimated by residual starch concentration assay at various temperatures. The test tubes were stored in a water bath at specific temperature (25, 30, 37, 45, 60, 70, 80 and 90°C).

The effect of the pH on the activity of native and entrapped enzyme was investigated by residual starch concentration assay, but in the presence of citric acid 0.1 M – Na_2HPO_4 0.2 M buffer ranged from pH 2.6 to 8 at room temperature.

Determination of kinetics parameters of native and immobilized enzyme: K_M and V_{max} were determined by measuring initial rates of Zulkowsky starch hydrolysis. Kinetics studies were conducted in citrate –phosphate buffer 0.15 M, pH 4.6, at 37°C in a 50 mL stirred jacketed batch reactor. The starch concentrations were 1.5 - 6.25 mg/mL. The reaction was started by addition of the enzyme (4 mL native enzyme and 20 mg immobilized enzyme) and 1 mL samples were collected every 2 minutes during the first 20 minutes of the reaction. Residual starch concentration assay (I_2/I^-) was used for analyze the samples [8].

RESULTS AND DISCUSSION

The influence of the pH on the activities of the native and immobilized α-amylase was studied in the pH range 2.6 – 8, at room temperature. The optimal pH for the native enzyme was 5.6. As a result of the immobilization process the optimal pH for the entrapped enzymes was shifted towards the alkaline side with approximately 2 units in the case of matrices based on TEOS and MTES:TEOS 1:1 and 2:1 respectively. In the case of the most hydrophobic matrix, MTES:TEOS 3:1, the optimal pH was 6 (Figure 1).

The effect of the temperature on the activities of the native and immobilized α-amylase was investigated in the 20 - 90°C range. As in the case of the pH dependence, the native enzyme presents a “bell-shaped” profile. The optimal temperature for the native enzyme was 60°C. In the cases of the matrices obtained from TEOS and MTES:TEOS 1:1 and 2:1, respectively, the optimal temperature is decreased with 15 and 23°C. The relative activities of α-amylase immobilized in matrix based on MTES:TEOS 3:1 are higher than

those of free enzyme over broader temperature ranges (Figure 2).

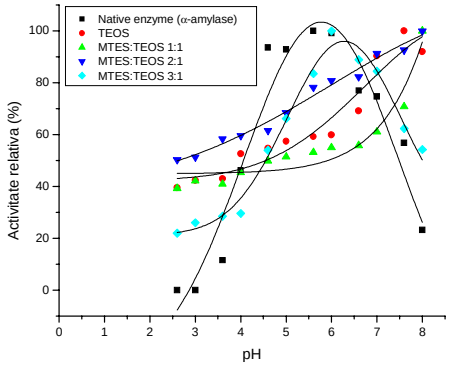

Figure 1. The effect of the pH on activity of immobilized and native α-amylase

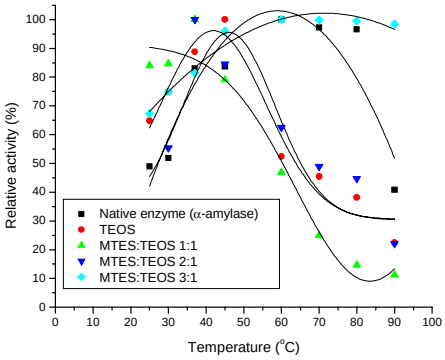

Figure 2. Temperature effect on activity of immobilized and native α-amylase

The kinetic parameters were estimated by fitting the experimental data to the Michaelis-Menten equation (Figure 3). The Michaelis constants (K_M) and maximum velocities (V_{max}) of the immobilized enzyme are smaller than those of the free enzyme are (Table 1). The highest K_M and V_{max} values were obtained in the case of MTES:TEOS 1:1 matrix and the lowest for TEOS matrix. However, the V_{max}/K_M ratio shows that the best catalytic efficiency, for those entrapped enzymes, was obtained in the case of TEOS.

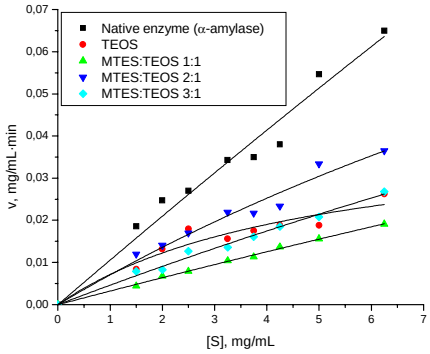

Figure 3. Initial rates (v) vs. substrate concentration ([S]) plots of native and immobilized α-amylase

Table I. Kinetic parameters for the native and immobilized α-amylase, estimated by fitting the experimental data to the Michaelis-Menten equation

Matrices	V_{max} mg/mL· min	K_M mg/mL	$V_{max} \cdot 100 / K_M$ min^{-1}
-	1.44	135.66	1.06
TEOS	0.04	4.92	0.81
MTES:TEOS 1:1	0.32	97.13	0.33
MTES:TEOS 2:1	0.17	23.56	0.72
MTES:TEOS 3:1	0.24	50.01	0.48

CONCLUSION

In the case of the α-amylase immobilized in matrices obtained from TEOS and MTES:TEOS 1:1 and 2:1, optimum pH was shifted to the basic side by two units and optimum temperature decreased with 15 and 23°C. α-Amylase immobilized in matrix based on MTES:TEOS 3:1 maintained the same optimum pH and temperature as the native counterpart.

The kinetic parameters of the immobilized enzyme are smaller than those of the free enzyme are. The V_{max}/K_M ratio indicates that matrix obtained from TEOS is the most favorable for the enzyme, with reference to the catalytic efficiency. As regards the hybrid matrices the highest V_{max}/K_M ratio was obtained in the case of MTES:TEOS 2:1.

In order to improve the sol-gel immobilized biocatalysts, one must combine organic, inorganic and biological components that work in synergy.

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